Su Ürünleri Dergisi	Cilt No.18/1	Özel Sayı	167 - 171	İzmir – Bornova 2001
J.Fish.Aquat.Sci.	Vol.18/1	Suppl.	167 - 171	İzmir – Bornova 2001

Evaluation of Some Biological Activities of *Chara hispida* L.

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Özet : *Chara hispida L.'nın Bazı Biyolojik Aktiviteleri Yönünden Değerlendirilmesi.* Chlorophyta divisiosuna dahil bir tür olan *Chara hispida* L. (Characeae), tatlı sularda ve bataklıklarda yetişen çok hücreli, dallanmış alglerdir. Kalsiyum karbonat bakımından oldukça zengin olan *Chara* türleri üzerinde yapılan çalışmalar, bunların gibberellik asit, peptit, protamin, sterol ve yağ asitleri içerdiklerini göstermiştir.

Deniz ve tatlı su alglerinden elde edilen birçok madde biyomedikal ve kozmetik alanda kullanıma girmiş veya girmek üzeredir. Ülkemizin deniz ve tatlı su kaynakları yönünden oldukça zengin olmasına rağmen, bu türler üzerinde çok az çalışma bulunmaktadır.

Bu araştırmada, daha önce üzerinde hiç biyolojik aktivite çalışması yapılmamış olan ve Salda Gölü'nden toplanan *Chara hispida* L. 'dan hazırlanan ekstrelerin antibakteriyel, antialgal aktiviteleri ile ihtiyotoksisite ve tuzlu su karidesine karşı toksisiteleri incelenerek elde edilen sonuçlar değerlendirilmektedir.

Anahtar Kelimeler : *Chara hispida*, Antialgal aktivite, Antibakteriyel aktivite, Tuzlu su karidesi, İhtiyotoksisite

Abstract : *Chara hispida* L. (Characeae), a species of Chlorophyta division, is a multicellular alga growing in fresh-waters and swamps. The studies on *Chara* species which are quite rich in calcium carbonate showed that these species contain gibberelic acid, peptide, protamine, sterol and fatty acids.

A lot of compounds obtained from marine and fresh-water algae have been used in biomedical area and cosmetic products. Although Turkey is quite rich in regard with marine and fresh-water resources, there are a few studies on aquatic species.

In this research, the results obtained by examining antibacterial, and antialgal activities along with ichthyotoxicity and toxicity against brine shrimp of the extracts prepared from *Chara hispida* L. on which no biological activity study has been carried out previously, collected from Salda Lake, are evaluated.

Key Words : *Chara hispida*, antialgal activity, antibacterial activity, Brine Shrimp, ichthyotoxicity

Introduction

As oceans cover 70 % of the earth, they constitute a great part of ecosystem which hosts numerous species of organisms. A lot of compounds obtained from marine and fresh-water algae have been used in biomedical area and cosmetic products. Although Turkey is quite rich in regard with marine and fresh-water resources, there are a few studies on aquatic species. *Chara hispida* L. (Characeae), a species of Chlorophyta division (Van den Hoek C. et al. 1995), is a multicellular alga growing in fresh-waters and swamps. The studies on *Chara* species which are quite rich in calcium carbonate showed that these species contain gibberelic acid, peptide, protamine, sterol and fatty acids. (Kazmierczak et al., 1999, Kazmierczak,1999, Maszewski., 1999, Khaliq-Uz-Zaman et al.,1998, Reynolds, and Wolfe,1984, Gosek and Kwiatkowska, 1991).

The biological activities screened in this study are important and useful in water ecology studies. For instance; the aquatic plant extracts or pure compounds isolated from these plants with antimicrobial activity have a possible role as defense against both microbial epibionts and larval settlement which is induced more favorably in the presence of bacterial film. Microorganisms and microalgae, the most common epibionts, might affect photosynthesis since clean leaves have a higher photosynthesis rates compared to heavily epiphytized ones. Antimicrobial agents produced by aquatic plants can act as fouling retardants. On the other hand, antialgal test helps establishing the interactions among marine organisms through the excretion of growth-inhibiting substances which have been suggested to play an important role in ecology.

In this study, the results obtained by examining antibacterial and antialgal activities along with ichthyotoxicity and toxicity against brine shrimp of the extracts prepared from *Chara hispida* L. of Salda Lake, on which no biological activity study has been carried out previously, are described.

Materials And Method

Collection and identification of the alga species Fresh samples of *C. hispida* L. were collected from Salda Lake, Burdur, Turkey in July, 2001. The material are stored freshly in formaldehyde solution at the Department of Biology, Faculty of Education, Gazi University, Ankara, Turkey.

Preparation of the crude extracts of C. hispida L.

The plant material was air-dried, powdered and weighed accurately. The materials were macerated with petroleum ether, chloroform, ethyl acetate and methanol, subsequently, for two days at room temperature. The extracts were filtered and concentrated to dryness under vacuum.

Test organisms

Gram-positive bacteria *Staphylococcus aureus* (DMST 4746) and *Bacillus subtilis* (DMST 5871), and gram-negative bacteria *Vibrio parahaemolyticus* (DMST 5665) and *Escherichia coli* (DMST 4212) used in antibacterial activity test were obtained from the culture collection of Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand. The microalgae *Nitzschia* sp. and *Chlorella* sp. were provided by

Faculty of Fisheries of Kasetsart University. Eggs of brine shrimp *Artemia salina* were purchased from the INVE company (Belgium) and hatched in the laboratory. Guppy fish *Lebistes reticulatus* used in the piscicidal activity test and prawn *Macrobranchium lancesteri* were scooped from the fish pond of Faculty of Fisheries of Kasetsart University, Thailand.

Biological activity assays

Antibacterial and antialgal activity by disk diffusion method, (Chan,1980, Harrison and Chan,1980, Tringali, 1991) ichthyotoxicity by using guppy fish *Lebistes reticulatus* and general toxicity tests against brine shrimp *Artemia salina* and the Riceland prawn *Macrobranchium lancesteri* de Man were carried out on the crude extracts of *C. hispida* according to the methods given in references.

Results and Discussion

According to the results we have obtained, petroleum-ether, chloroform and methanolic extracts of *C. hispida* showed moderate activity against *A. salina* at 100 mg/L concentration. In this test, the most active extract was the ethyl acetate extract, killing 5 larvae of *A. salina*. None of the extracts had antialgal activity. In the antibacterial test, chloroform and ethyl acetate extracts of *C. hispida* displayed activity only against *B. subtilis* at 500 µg/disk, causing 17 mm (in diameter) inhibition zone. Ethyl acetate extract of *C. hispida* possessed strong ichthyotoxicity against guppy fish (*Lebisdes reticulatus*) at 100 and 500 µg/L concentrations, killing one fish for each concentration within 4 hours. The same extract was also toxic to Riceland prawn *Macrobranchium lancesteri* at 500 µg/L (Table 1).

As mentioned previously, nature has provided aquatic organisms with a number of noxious subtances for both offensive and defensive purposes since they are sessile organisms and lack obvious mechanical devise. (Tringali, 1991, Meyer et at, 1982, Sam, 1993, Lincoln et al.,1996, Wongrat, 1996) In addition, the study of marine toxins has the utilitarian purpose of discovering compounds which are of potential therapeutic value, or can be used as pharmacological tools in biological and medical sciences. Among the extracts with various polarity of the fresh-water alga *C. hispida*, ethyl acetate extract is the most active one and deserves further phytochemical investigation in order to find out its active components. To best of our knowledge, this is the first study on biological activities of *C. hispida*.

Table 1. Biological activity results of the fresh-water alga *Chara hispida.*

The extracts	Toxicity against *Artemia salina*		Toxicity against *Macrobranchium lancesteri*	Ichthyotoxicity	
	10 µg/L	100 µg/L	500 µg/L	100 µg/L	500 µg/L
CH-PE[a]	-	2	-	-	-
CH-$CHCl_3$[b]	-	2	-	-	-
CH-EtOAc[c]	-	5	1	1	1
CH-MeOH[d]	-	2	-	-	-

[a] Petroleum-ether extract of *C. hispida*, [b] Chloroform extract of *C. hispida*,
[c] Ethyl acetate extract of *C. hispida*, [d] Methanolic extract of *C. hispida*

References

Chan, A.T., Amdersen, R.J., Le Blanc, M.J., Harrison, P. J., Algal plating as a tool for investigating allelopathy among marine macroalgae, *Marine Biol.*, 59, 7-13, 1980.

Gosek, A., Kwiatkowska, M.: Cytochemical studies on the antheridial mucilage and changes in its concentration and amount during the spermatogenesis in *Chara vulgaris* L., *Folia Histochemica et Cytobiologica*, 29(3), 91-99, 1991.

Harrison, P.G., Chan, A.T., Inhibition of the growth of microalgae and bacteria by extracts of eelgrass (*Zostera marina*) leaves. *Marine Biol.* 61, 21-26, 1980.

Kazmierczak, A., Kwiatkowska, M., Poplonska, K.: GA (3) Content in antheridia of *Chara vulgaris* at the proliferative stage and in spermiogenesis estimated by capillary electrophoresis, *Folia Histochemica et Cytobiologica*, 37(1), 49-52, 1999.

Kazmierczak, A.: Determination of GA(3) in *Chara vulgaris* by capillary electrophoresis system, *Acta Physiologiae Plantarum*, 21(4), 345-348, 1999.

Khaliq-Uz-Zaman, S.M., Shameel, M., Shameel, M., Leghari, S.M., Ahmad, V.U.: Bioactive Compounds in *Chara corallina* var. *wallichii* (A. Br.) R.D. Wood (Charophyta), *Pakistan Journal of Botany*, 30(1), 19-31, 1998.

Lincoln, R.A., Strupinski, K., Walker, J.M., The use of *Artemia* nauplii (brine shrimp larvae) to detect toxic compounds from microalgal cultures, *Int. J. Pharmacognosy*, 34, 384-389, 1996.

Maszewski, J., Maszewska, M., Polit, J.: Effects of antheridial peptides derived from *Chara* on cell cycle-regulated processes in human and yeast cells, *Folia Histochemica et Cytobiologica*, 37(3), 209-217, 1999.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., Brine shrimp: A convenient general bioassay for active plant constituents, *Planta Medica*, 45, 31-34, 1982.

Reynolds, W.F., Wolfe, S.L.: Protamines in plant sperm, *Exp. Cell. Res.*, 152(2), 443-448, 1984.

Sam, T. W., Bioactive natural products, 441-456, CRC Press, New York, 1993.

Tringali, C., Modern methods for plant analysis, p. 253-290, Springer-Verlag, Berlin, 1991.

Van den Hoek, C., Mann, D.G., Jahns, H.M., Algae, an introduction to phycology, Cambridge Univ. Pres., 1995.

Wongrat, L. Handbook on plankton culture, p. 12-113, Publications of Faculty of Fisheries, Kasetsart University, Bangkok, 1996.